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Interferon- γ -Induced Oligodendrocyte Cell Death: Implications for the Pathogenesis of Multiple Sclerosis

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ABSTRACT

Background: The histopathology of multiple sclerosis (MS) is characterized by a loss of myelin and oligodendrocytes, relative preservation of axons, and a modest inflammatory response. The reasons for this selective oligodendrocyte death and demyelination are unknown. **Materials and Methods:** In light of the T lymphocyte and macrophage infiltrates in MS lesions and the numerous cytokines these cells secrete, the direct influence of cytokines on survival of cultured oligodendrocytes and sensory neurons was investigated. Expression of cytokines in vivo was determined by immunolabeling cryostat sections of snap-frozen tissue containing chronic active lesions from four different patients. The samples were also analyzed for the presence of apoptotic nuclei by in situ labeling of 3'-OH ends of degraded nuclear DNA.

Results: The results showed: (i) interferon- γ (IFN γ) to be a potent inducer of apoptosis among oligodendrocytes in vitro and that this effect can be reversed by leukemia inhibitory factor (LIF); (ii) IFN γ has a minimal effect on the survival of cultured neurons; (iii) IFN γ at the margins of active MS plaques but not in unaffected white matter; (iv) evidence for apoptosis of oligodendrocytes at the advancing margins of chronic active MS plaques. **Conclusions:** Injury to a substantial number of oligodendrocytes in MS is the result of programmed cell death rather than necrotic cell death mechanisms. We postulate that IFN γ plays a role in the pathogenesis of MS by activating apoptosis in oligodendrocytes.

INTRODUCTION

The pathologic hallmark of multiple sclerosis (MS) is a loss of oligodendrocytes, demyelination, and modest inflammation with relative preservation of neuronal elements (1-4). What allows for this remarkable specificity? A simple explanation for the specificity is that MS is a primary degenerative disease of oligodendrocytes. Death of oligodendrocytes would result in the release of encephalitogenic proteins which

evoke an immune response as an epiphenomenon rather than the disease beginning with self-reactive T lymphocytes or antibodies (5). Adrenoleukodystrophy is an example of a disease in which death of oligodendrocytes yields a secondary inflammatory response (6). Another hypothesis is that focal damage to the integrity of the blood-brain barrier (BBB) may allow passage of molecules specifically toxic to oligodendrocytes (4,7,8). For example, oligodendrocytes have complement receptors, and complement, which is normally excluded by the intact BBB, could pass through a disrupted BBB to injure oligodendrocytes (8-10). The most studied current theory is that there is primary immune dysregulation in

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MS which unleashes an immune response that specifically targets oligodendrocyte/myelin antigens (11,12). It has been proposed that the pathogenic autoimmune response in MS is mainly cell mediated (11,12). However, the published evidence in support of this hypothesis is characterized by inconsistencies with regard to the specific T lymphocyte subsets present in MS lesions (13-15). Furthermore, macrophage/activated microglia, not T lymphocytes, may be the first immigrant cells to appear in active MS lesions (1-3,16).

Cell death is currently divided morphologically and mechanistically into the broad categories of necrotic cell death versus programmed or apoptotic cell death. Apoptosis is characterized by early condensation of chromatin, nuclear shrinkage, and fragmentation of DNA into nucleosome-sized components with preservation of the plasmalemma and mitochondria (17,18). Necrotic cell death is marked by early disruption of the plasma membrane, swelling of cytosol and mitochondria with nuclear changes occurring late (17,18). The molecular mechanisms of programmed cell death in mammalian cells are only currently being elucidated (18,19). Cell death in pathologic conditions can be either necrotic or apoptotic (20). During normal mammalian development the numbers of oligodendrocytes are in part controlled by programmed cell death, presumably as a result of competition for growth and survival factors (21). Oligodendrocyte survival is promoted by members of the leukemia inhibitory factor (LIF) family of growth factors and by insulin-like growth factor-1 (21,22).

In searching for a factor that might directly mediate oligodendrocyte injury in MS we set the following criteria: (i) it should injure isolated oligodendrocytes *in vitro* with cellular changes similar to those observed in MS lesions; (ii) it should be present in active recent and chronic active MS lesions; (iii) it should not affect or minimally affect cultured neurons. We have found that pure recombinant rat interferon- γ (IFN γ) kills rat oligodendrocytes and that this effect can be inhibited by anti-rat-IFN γ antibodies. Oligodendrocytes exposed to IFN γ have pyknotic nuclei and degradation of DNA into nucleosome-sized fragments consistent with apoptotic rather than necrotic cell death. IFN γ -treated oligodendrocytes can be partially rescued by treatment with LIF. However, IFN γ does not injure cultured neurons. In fresh frozen autopsy tissue from MS patients, IFN γ was localized to the advancing margins of chronic active lesions

as previously described by Traugott and Lebon (23). Furthermore, apoptotic cells were found at the advancing margins of chronic active MS lesions, many of which were oligodendrocytes. On the basis of these results we propose that IFN γ may be one of the factors that mediate the loss of oligodendrocytes in MS.

MATERIALS AND METHODS

Antibodies

The galactosylcerebroside recognizing O1 antibody was generated by Dr. M. Schachner and colleagues (24), and the hybridoma was obtained from Dr. Stephen Pfeiffer (University of Connecticut, Farmington). Mouse anti-rat-IFN γ (Gibco-BRL, Gaithersburg, MD, U.S.A.) was used at a concentration of 2 neutralizing units per unit of IFN γ for *in vitro* blocking experiments to demonstrate specificity. Anti-human-IFN γ (Genzyme, Cambridge, MA, U.S.A.) was used at a concentration of 5 μ g/ml for immunohistochemistry of MS lesions. Mouse anti-rat myelin-oligodendrocyte-glycoprotein (MOG) was used at a dilution of 1:200 (provided independently by Dr. Charissa Dyer and Dr. Minetta Gardinier). FITC-conjugated goat anti-rabbit antibodies, and FITC-conjugated goat anti-mouse antibodies were obtained from Sigma (St. Louis, MO, U.S.A.). Peroxidase-conjugated goat anti-mouse IgG (TAGO) was used at a dilution of 1:100.

Cytokines

Because of species specificity, all interferons used for *in vitro* experiments on rat glial cells were themselves from rat. Rat IFN γ was recombinant (Gibco-BRL), and unless otherwise stated, was used at a concentration of 50 U/ml. Recombinant mouse tumor necrosis factor α (TNF α) (Gibco-BRL), which is active on rat cells, was used at a concentration of 100 ng/ml.

Histochemistry

Ten-micrometer cryostat sections of snap-frozen tissue from MS patients were mounted on gelatin-coated glass slides. Endogenous peroxidases were quenched by 30-min incubation with 3% H₂O₂ in PBS and 0.3% Triton X-100. Nonspecific sites were blocked with 5% milk in 0.3% Triton X-100 in PBS. Sections were incubated with primary antibodies overnight at 4°C and with per-

oxidase-conjugated secondary antibodies for 3 hr at room temperature. Peroxidase activity was detected by reaction with DAB and H_2O_2 . Apoptotic nuclei were labeled in situ as previously described (25). Sections were permeabilized in ethanol:acetic acid (2:1), washed then incubated for 1 hr at 37°C with terminal deoxynucleotidyl transferase and Digoxigenin-dUTP. Labeled DNA was identified with peroxidase-conjugated goat anti-digoxigenin antibodies followed by reaction with H_2O_2 and DAB (Oncor, Gaithersburg, MD). Prior to incubation with anti-MOG antibodies for double labelling, remaining peroxidase activity was quenched with 3% H_2O_2 .

Cell Culture

Oligodendrocyte progenitors were isolated from the forebrains of 1-day-old rats using a previously described method (26), with slight modifications (27). Cultures seeded with cells dissociated from P1 forebrains contain oligodendrocyte-type II astrocyte (O2A) progenitors, endothelial cells, microglia, neurons, and type II astrocytes, all of which grow on a monolayer of type I astrocytes. Following detachment of progenitors from the crude mixtures by a 15-hr 180 rpm shake, recovered cells, predominantly O2A progenitors, type II astrocytes, and microglia, were subjected to three successive incubations in 100 mm tissue culture dishes. This nonspecific panning enriched for O2A progenitors, because astrocytes, endothelial cells, and microglia adhere more rapidly to tissue culture plastic than do O2A progenitors. In addition, leucine methyl ester was added to the cells during panning, and similar to previous reports, this procedure reduced microglia to less than 0.5%, and astrocytes to less than 7% of the plating mixture (27). Viable cells remaining in suspension were then plated onto polyornithine-coated 25-mm glass coverslips in Dulbecco's modified Eagle's medium (DMEM) plus 5% fetal calf serum (Day 0). After 3 hr, plating medium was replaced with defined medium for oligodendrocytes (DM_{ol}), which favors survival and growth of cells in the oligodendrocyte lineage or DM_{ol} plus PDGF-AA and bFGF to maintain cells as O2A progenitors. DM_{ol} contains DMEM (Gibco-BRL), 0.5% recrystallized BSA, 5 μ g/ml insulin, 100 μ M putrescine, 50 μ g/ml transferrin, 5 ng/ml selenium, 30 nM triiodothyronine, 20 nM progesterone, and 10 ng/ml D-biotin (Sigma). Prepared as described, cultures O2A cells or oligodendrocytes contained approximately 10% astrocytes as de-

termined by GFAP staining, and approximately 0.5% ameboid microglia as determined by staining with dil-low-density lipoprotein (LDL).

Ameboid microglia were cultured as previously described (27). Mixed glial cultures were agitated on a rotary shaker for 15 hr at 37°C and 180 rpm. The cell suspension obtained was plated into new tissue culture flasks for 3 hr, after which flasks were gently rotated and non-adherent cells discarded. Adherent cells were removed by treatment with trypsin and vigorous shaking, and transferred to new tissue culture flasks containing fetal bovine serum (FBS) (15% final volume) for another three-hour interval. Non-adherent cells were removed, and adherent cells were composed of 95% dil-LDL positive microglia.

MTT AS A MEASURE OF CELL VIABILITY. Viability of cells was assessed using MTT, a tetrazolium salt cleaved with dehydrogenases of mitochondria to a water insoluble purple formazan (28,29). Three hours prior to the end of the incubation time the cultures were incubated with MTT to label living cells. Fifteen minutes prior to fixation the cultures were incubated with the O1 antibody to label surface galactocerebroside. At the end of the incubation time cultures were fixed with 4% paraformaldehyde followed with FITC-conjugated second antibody. Viable oligodendrocytes were defined by colabeling of MTT staining and O1 immunoreactivity.

DNA FRAGMENTATION. Oligodendrocytes were cultured as described above at a density of 5×10^4 cells/cm² in 60-mm tissue culture dishes. Cultures were treated for 48 hr with either PBS (control), and both 24 and 48 hr with rat IFN γ at 50 U/ml, then washed twice with ice-cold PBS (pH 7.4), and solubilized in lysis buffer (5 mM Tris, pH 7.4, 20 mM EDTA, 0.5% Triton X-100) as previously described (30). The supernatants from solubilized cells were extracted twice with Tris-buffered phenol (pH 8.0), then once with chloroform:isoamyl alcohol (24:1 v/v). Soluble nucleic acids were ethanol precipitated, resuspended in Tris-EDTA and digested with RNase A (50 μ g/ml) at 37°C for 30 min. DNA was resolved on 1.2% agarose gels, transferred to a Gene-screen Plus membrane (DuPont, Boston, MA), probed with random primed radiolabeled *Rsa*I digested genomic rat DNA, and washed for 30 min at 50°C with 0.1X SSC and 0.1% SDS. Membranes were exposed to film for 12 hr with intensifying screens.

RESULTS

Oligodendrocyte-type II astrocyte (O2A) progenitor cells are glial progenitors that can be isolated from 1-day-old rat forebrain (26) and derive their name from a bipotential nature in vitro (31). O2A cells are characterized by a distinct bipolar morphology and immunoreactivity with the A2B5 antibody (31). When cultured in a defined medium containing insulin or members of the LIF family of ligands (21,22), these cells pass through a pro-oligodendroblast stage (32) and differentiate into oligodendrocytes (31,32) characterized by extensive lace or sheet-like processes and expression of surface galactosylcerebroside recognized by the O1 antibody (33); when cultured in the presence of serum they differentiate into type II astrocytes (31) marked by stellate morphology and staining for glial fibrillary acidic protein (GFAP). The O2A cell can be perpetuated as a noncommitted bipotential progenitor when grown in the presence of PDGF-AA and bFGF (34). We studied the effects of recombinant rat IFN γ on survival of rat O2A progenitors and oligodendrocytes derived from them. Because IFN γ is a potent activator of blood borne monocytes (35,36), we sought to eliminate indirect effects by depleting the cultures of closely related amoeboid microglia to less than 0.5% of the total number of cells (as determined by the presence of the high-affinity LDL receptor detected with dil-LDL [27]). Viability of O2A progenitors and oligodendrocytes was determined using the conversion of water soluble MTT into an insoluble purple precipitate by mitochondrial dehydrogenases (28,29). MTT-positive cells were also phase bright and excluded trypan blue.

Exposure of cultures to rat IFN γ (50 U/ml) for 2 days led to the death of 75% of O2A progenitors and oligodendrocytes (Fig. 1A). O2A progenitors are motile, and, prior to death, they migrated into clusters of 6–10 cells. The effect of IFN γ on O2A progenitors and oligodendrocytes was dose dependent, with an EC₅₀ of ~15 U/ml (Fig. 1B). After 4 days of exposure to IFN γ , most of the cells had begun to demonstrate late morphologic changes of death, such as degeneration of processes into vacuoles (Fig. 1C).

It has been reported that TNF α , at concentrations of 100 ng/ml, may be toxic to oligodendrocytes (29,37). In cultures enriched for oligodendrocytes, with microglia accounting for less than 0.5% of the cells, we find no evidence for TNF α (100 ng/ml)-induced oligodendrocyte cell

death (Fig. 1C). Our preparation of TNF α was active as it induced nitric oxide synthase activity in amoeboid microglia.

While we depleted oligodendrocyte cultures of microglia to approximately 0.5% of the cells, it was a remote possibility that IFN γ was acting through a few contaminating microglia. To test this possibility, we performed the following experiments. First, we added equivalent numbers of amoeboid microglia to cultures of oligodendrocytes and assessed the viability of oligodendrocytes with and without IFN γ treatment. As noted in Fig. 2A, there was no significant difference in the percentage of oligodendrocyte killing by IFN γ in the presence or absence of amoeboid microglia.

It has been proposed that oligodendrocytes are susceptible to nitric oxide-mediated injury (38). Indeed, rat microglia express inducible nitric oxide synthase (NOS) in response to cytokines and lipopolysaccharide (LPS). The presence of NOS can be detected by the NADPH diaphorase reaction which results in an insoluble purple-black precipitate (39,40). IFN γ and LPS induce NOS activity in amoeboid microglia, as determined by NADPH diaphorase staining (Fig. 2B). To assess the effects of nitric oxide on oligodendrocytes, we utilized tissue culture inserts with a highly permeable surface which can be placed in close apposition to cells grown in a tissue culture well. Microglia were cultured upon 0.45- μ m high-density (1.6×10^8 pores/cm²), porous inserts, treated with IFN γ or control solution for 24 hr, then washed five times with 30-min incubations in fresh culture medium. Inserts were then placed 1 mm above cultures of oligodendrocytes in the presence of nitric oxide scavengers (reduced hemoglobin, 500 μ M), the NOS inhibitor N^G-methyl-L-arginine (500 μ M) both during washes as well as coculture, anti-IFN γ antibodies (200 neutralizing units/ml), or control buffer. After 48 hr of coculture we observed a 30%–40% decrease in the number of viable oligodendrocytes in all conditions except with anti-IFN γ antibodies (Fig. 2C). The absence of reversal in the presence of nitric oxide and NOS inhibitors suggested to us that nitric oxide was not toxic to oligodendrocytes, but rather that IFN γ was either inducing synthesis of IFN γ in, or that it was adhering to microglia, being released during coculture and killing oligodendrocytes by a direct pathway. To assess these possibilities, we performed the same experiments as described for Fig. 2C except this time using fibroblasts in place of microglia. To our surprise, we obtained similar

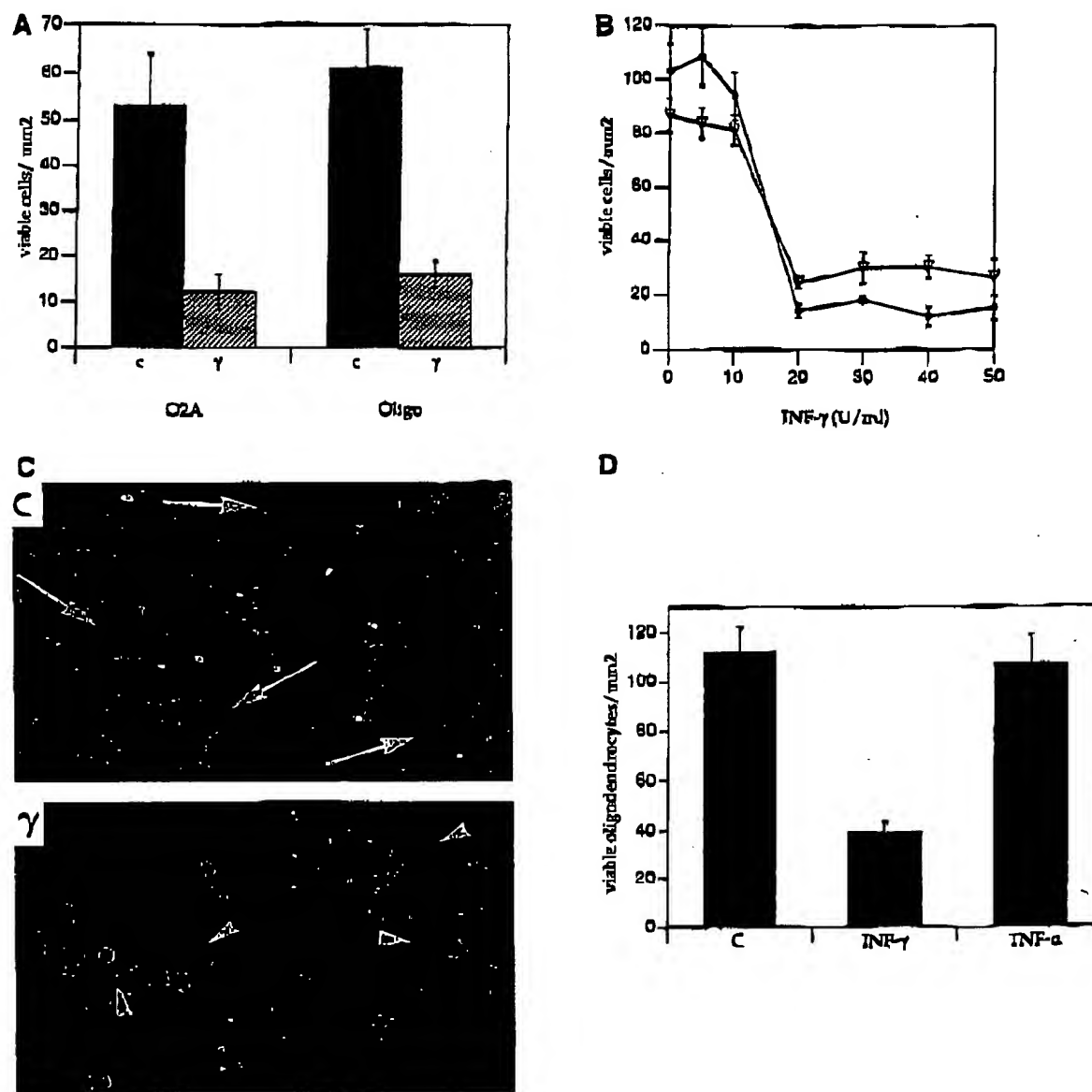


FIG. 1. Effect of IFN- γ on survival of O2A progenitors and oligodendrocytes.

(A) Number of viable O2A progenitor cells or oligodendrocytes as a function of a 48-hr exposure to control buffer or IFN- γ (γ). $p < 0.01$ for all comparisons. (B) O2A progenitor cell (●) and oligodendrocyte (▽) survival as a function of IFN- γ concentration. (C) Micrographs of oligodendrocytes treated for four days with control buffer or IFN- γ . Cells were stained for surface galactosylcerebroside with the OL antibody. Viable oligodendrocytes with characteristic sheet-like processes (arrows) are shown in the control (c) panel. Late morphologic changes of cell death including degradation of processes into vacuoles are noted in the cultures treated with IFN- γ (γ) for 4 days (arrowheads). (D) Comparison of the effects of IFN- γ and TNF- α on oligodendrocyte survival.

results (Fig. 2D), suggesting that IFN- γ was adhering to cells on the inserts since fibroblast do not synthesize IFN- γ . Taken with the data in Fig. 1, our in vitro work supports direct killing of oligodendrocytes by IFN- γ .

A major feature of the histopathology of MS is that there is little neuronal injury or axonal loss (1-3). We thus investigated the effects of rat IFN- γ on the viability of sensory neurons in vitro. Neurons isolated from E15 rat dorsal root ganglia

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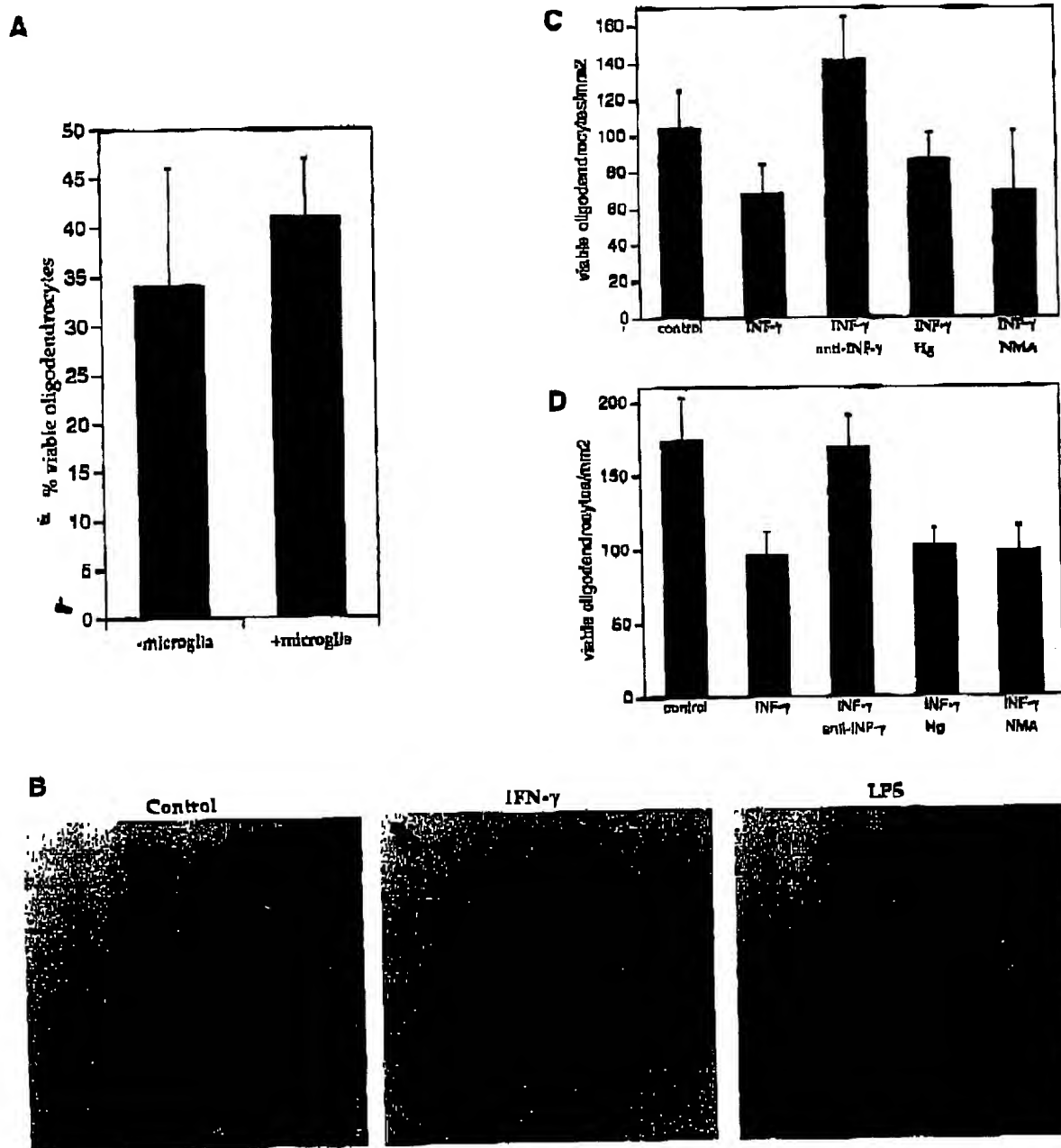


FIG. 2. IFN γ -mediated oligodendrocyte death is a direct effect and not mediated through microglia or by nitric oxide

(A) Number of viable oligodendrocytes in cocultures of oligodendrocytes and amoeboid microglia treated with IFN γ or PBS for 48 hr. Oligodendrocytes and microglia were plated together each at a density of 10,000 cells/mm² on glass coverslips. Cocultures were treated with IFN γ or PBS. (B) NADPH diaphorase staining of rat amoeboid microglia exposed to PBS (control), IFN γ , or LPS for 24 hr. NADPH diaphorase positive microglia stain intensely purple-black. (C) Number of viable oligodendrocytes 48 hr after coculture 1 mm beneath microglia containing inserts pretreated with IFN γ or PBS. Amoeboid microglia were cultured on membranes with 1.6×10^6 pores/cm² and an average pore size of 0.45 μ m. After treatment with IFN γ or PBS for 24 hr, inserts were washed with five 30-min incubations in fresh culture medium. Washed inserts were then placed over coverslips of oligodendrocytes in the presence of PBS, anti-IFN γ antibodies, hemoglobin (Hg) or N^G-methyl-L-arginine (NMA). (D) Number of viable oligodendrocytes 48 hr after coculture 1 mm beneath primary rat fibroblast containing inserts under conditions described above for Panel C.

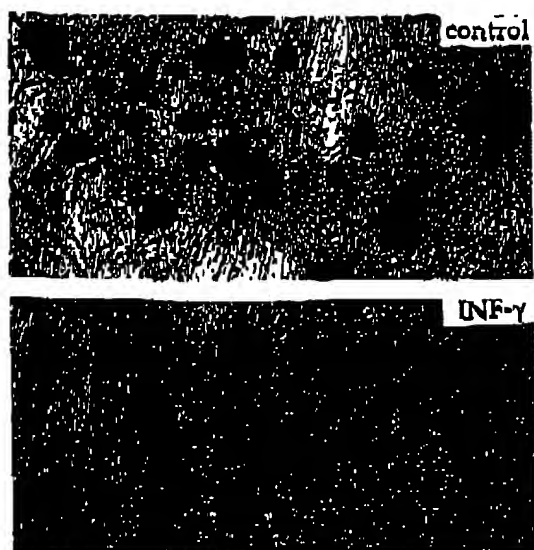


FIG. 3. Effect of IFN γ on the survival of sensory neurons

DRG neurons were cultured in the presence of NGF on a polyornithine/laminin substratum. After 2 weeks, *in vitro* cultures were exposed to control solution, or IFN γ for 7 days. Viability was assessed with MTT conversion to an insoluble purple precipitate shown above. There is a small decrease in the number of viable sensory neurons, which does not reach a statistical significance (controls, 126 ± 16 versus IFN γ , 103 ± 9.7) morphology and neuritic outgrowth from IFN γ treated neurons is indistinguishable from controls. Bar = 10 μ m.

were cultured for 2 weeks in the presence of nerve growth factor (NGF) (41). Contaminating fibroblasts and Schwann cells were eliminated by treatment of the cultures with 5-fluorodeoxyuridine as previously described (41). IFN γ did not significantly influence the viability of this cell population (Fig. 3).

We investigated the ability of concurrent treatment with human LIF at a concentration of 1000 U/ml to protect oligodendrocytes from IFN γ (50 U/ml)-mediated cell death. LIF increased the number of viable oligodendrocytes over cultures treated with IFN γ alone (Fig. 4). The effect of IFN γ was also blocked with pretreatment by a monoclonal anti-rat-IFN γ antibody (Fig. 4).

Oligodendrocytes treated for 18 hr with IFN γ have pyknotic nuclei with preservation of the plasmalemma suggestive of apoptosis (Fig. 5A). Another feature frequently associated with apoptosis is degradation of DNA into nucleosome-sized fragments, which can be visualized as DNA

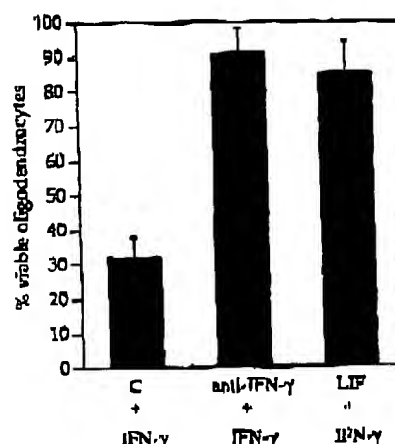


FIG. 4. Reversal of IFN γ -induced oligodendrocyte death by human LIF (1000 U/ml) and anti-rat-IFN γ antibodies

Concentrations used were determined to be those which generated a maximal effect in dose-dependent assays (not shown). All cultures including control (c) received rat IFN γ (1000 U/ml) plus the indicated reagents.

ladders when resolved by agarose electrophoresis (42). We find that IFN γ -treated O2A cells and oligodendrocytes demonstrate degradation of DNA into nucleosome-sized fragments consistent with apoptosis (Fig. 5B).

Degraded nuclear DNA of apoptotic cells can also be detected *in situ* by terminal labeling of the 3'-OH ends with digoxigenin-dUTP in the presence of terminal deoxynucleotide transferase (25). Tailed 3'-OH groups are identified with peroxidase-conjugated antibodies directed against digoxigenin and subsequent reaction with DAB and H $_2$ O $_2$. This method was used to identify apoptotic cells, and staining with a monoclonal antibody against the MOG was used to label oligodendrocytes (43). Snap-frozen tissue containing four chronic active lesions from four different patients were obtained at autopsies that were performed between 4 and 12 hr after death (see Acknowledgments). These samples were studied, and all showed results similar to those in Figs. 6 and 7. The advancing margins of chronic active MS plaques, but not unaffected white matter, contain numerous pyknotic, apoptotic nuclei within MOG labeled cells (Fig. 6 a and b). The labeled nuclei are not clustered in a contiguous mass of dead cells as in necrosis but rather are scattered amongst normal cells throughout the advancing margin of the lesion, a finding consis-

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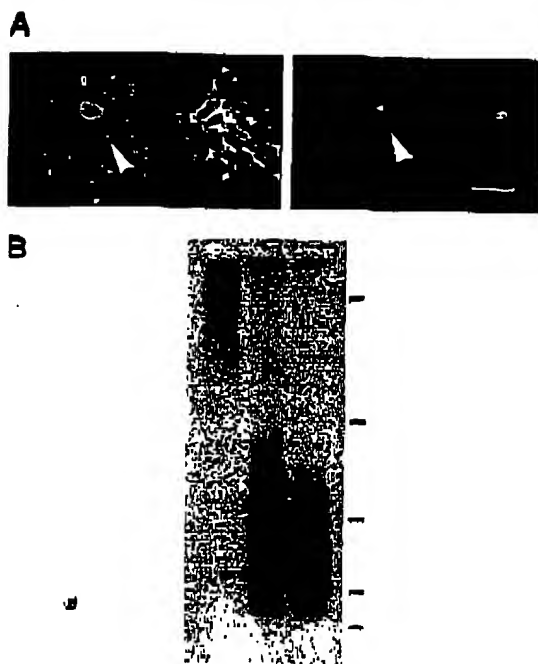


FIG. 5. IFN γ -induced glial cell death is apoptotic

(A) IFN γ treated oligodendrocytes have pyknotic nuclei. Oligodendrocytes were treated with IFN γ for 18 hr and then stained with the O1 antibody detected with FITC-conjugated goat anti-mouse IgM (left panel). Nuclei were counterstained with Hoechst dye (right panel). The figure shows the same field of an IFN γ -treated culture. Arrow head indicates a cell with normal morphology with O1 staining but a pyknotic nucleus with Hoechst stain (bar = 10 μ m). (B) DNA fragmentation in IFN γ treated cultures. Oligodendrocytes were cultured as described in the methods section. Beginning on Day 2 of culture, cells were treated with PBS for 48 hr (Lane 1) or IFN γ for 24 and 48 hr (Lanes 2 and 3, respectively). Soluble DNA fragments were isolated and resolved on a 1.2% agarose gel, transferred to a Gene Screen Plus membrane, and probed with 32 P-labeled rat genomic DNA. The membrane was exposed to film for 12 hr and the autoradiograph is shown. Bars at right represent the following sizes in base pairs from top to bottom: 9416, 2322, 872, 310, and 192.

tent with apoptosis (17–20). Nuclei in adjacent unaffected white matter (Figs. 6 and 7) and normal controls (not shown) do not label with the terminal transferase technique.

Our *in vitro* results suggest to us that IFN γ could play a role in the pathogenesis of diseases that entail death of oligodendrocytes. Therefore, we searched for IFN γ in snap-frozen tissue from autopsies of MS patients. Using a monoclonal antibody raised against recombinant human

IFN γ we find the cytokine at the advancing margins of active MS plaques but neither in adjacent unaffected white matter nor in the chronic portion of the plaques (Fig. 6 d and e). The IFN γ is within cells with the morphology of macrophages or activated microglia (Fig. 6d).

DISCUSSION

We have shown that IFN γ induces programmed death of oligodendrocytes and their precursors *in vitro*. Furthermore, we and others (23) find IFN γ in active MS lesions where we now find evidence for apoptosis of oligodendrocytes. Taken together, our data support the idea that IFN γ may play a role in the pathogenesis of MS by inducing apoptosis of oligodendrocytes.

The antiviral effects of IFN γ prompted its use in clinical trials in MS (44). Patients who received IFN γ had an increased frequency and severity of attacks (44,45). Furthermore, a rise in cerebrospinal fluid (CSF) IFN γ levels precedes exacerbations of MS (46–51). It has been suggested that the influence of IFN γ on the clinical course of MS is mediated through its effects on cells of the immune system (52–55), or through induction of MHC class II antigens on oligodendrocytes (56). Our results suggest that the detrimental effects of IFN γ on the course of MS may, at least in part, arise from direct toxicity to oligodendrocytes themselves. In fact, IFN γ has also been shown to potentiate demyelination in Sprague-Dawley rats mediated by anti-MOG antibodies (55).

In addition to our results with IFN γ , glutamate has been shown to injure oligodendrocytes *in vitro* (57). Oligodendrocytes and O2A cells have well described glutamate receptors (58,59); however, glutamate-mediated toxicity occurs in many neuronal populations and is not likely to result in a specific oligodendrocyte death as seen in MS. It has been postulated that complement plays a role in the pathogenesis of multiple sclerosis (4,8,9), and complement alone is indeed toxic to oligodendrocytes *in vitro* (8,9). However, this theory would predict that any disruption of the blood brain barrier should result in injury to oligodendrocytes as an innocent bystander, which does not seem to be the case.

We do not observe a toxic effect of TNF α on oligodendrocytes *in vitro* as previously reported (37,40). This difference in results might arise from the culture systems used. Investigators who have found an effect of TNF α on oligodendrocytes have used either an O2A-like cell line, CG4,

FIG. 6. Identification of apoptotic oligodendrocytes and IFN γ -positive cells at the margins of chronic active MS lesions

Cryostat sections of fresh frozen tissue from MS patients (17) were processed for colabeling apoptotic nuclei (brown) and MOG (purple/black). (a and b), human IFN γ (d and e), and oil-O-red to identify active phagocytic cells (c). Panels a, d show a chronic active MS lesion, whereas Panel e shows a region of unaffected white matter from the same patient. The tissue was removed at autopsy performed 4 hr after death and frozen. Some freezing artifacts are apparent in the sections.

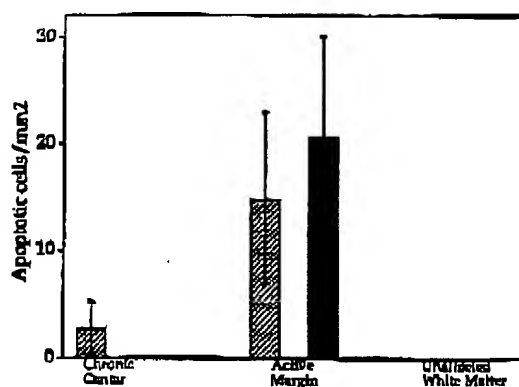
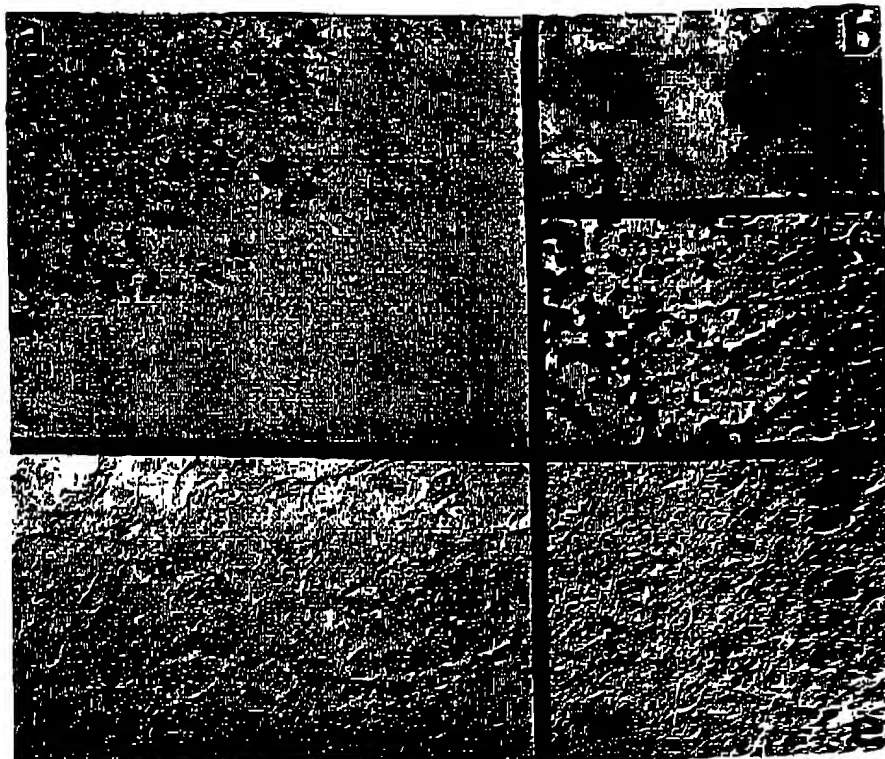


FIG. 7. Quantitation of apoptosis in the center and at the margin of the chronic active MS lesion shown in Fig. 6

Ten nonoverlapping 200X fields that comprised approximately half of the area occupied by the chronic center of the lesion and eight nonoverlapping contiguous 200X fields that comprised the majority of area occupied by the active margin were averaged. In addition, 10 random fields within unaffected white matter were counted. Apoptotic oligodendrocytes (filled bars) and apoptotic cells which did not colabel for the oligodendrocyte-specific protein MOG (hatched bars) were counted.

which may respond differently than primary O2A cells, or primary cultures from which microglia were not eliminated (37,39,40). Other laboratories have also failed to observe a direct toxic effect of TNF α on oligodendrocytes (38). Cerebral malaria is an example of a disease where TNF α is present in high concentration and the blood-brain barrier is disrupted (40). However, there is no specific injury to the oligodendrocyte-myelin unit in cerebral malaria, and thus it seems that TNF α is not always toxic to oligodendrocytes. This is not to say that in the context of other cytokines present in MS lesions TNF α would not injure oligodendrocytes.

The effects of IFN γ on oligodendrocytes were partially reversed with pretreatment or concurrent treatment with LIF. It is of interest that IFN γ , which adversely affected the outcome of MS, is also a potent inducer of oligodendrocyte death *in vitro* (45,61,62).

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